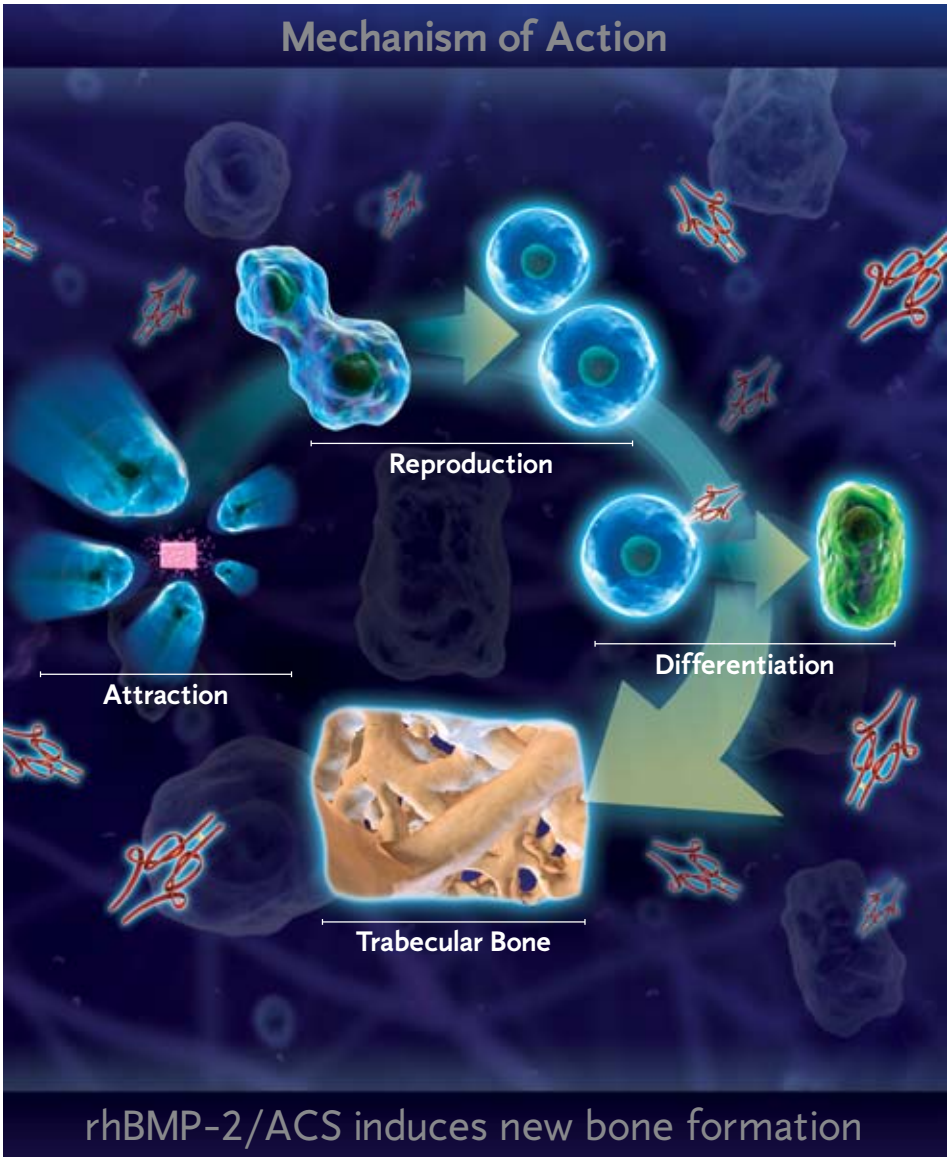
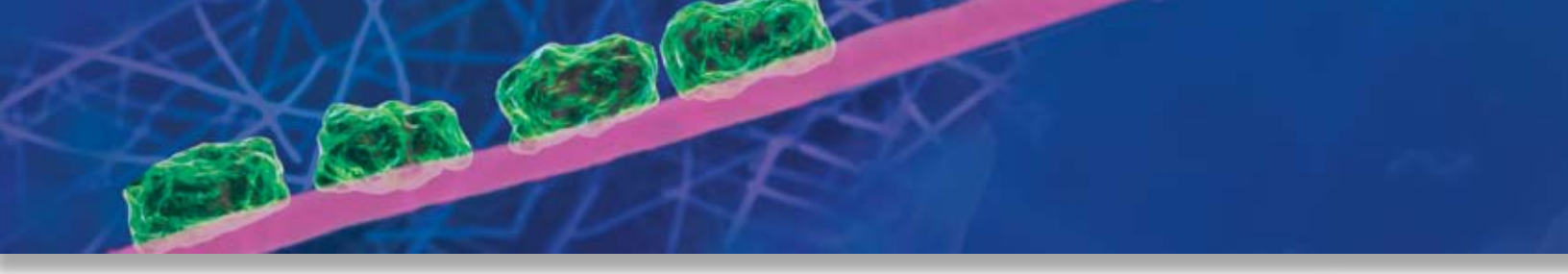
Medtronic
SOFAMOR DANEK

rhBMP-2/ACS

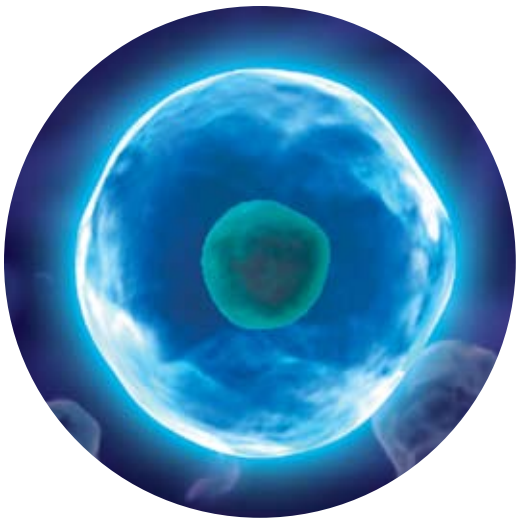
Understanding the Mechanism of Action[1]



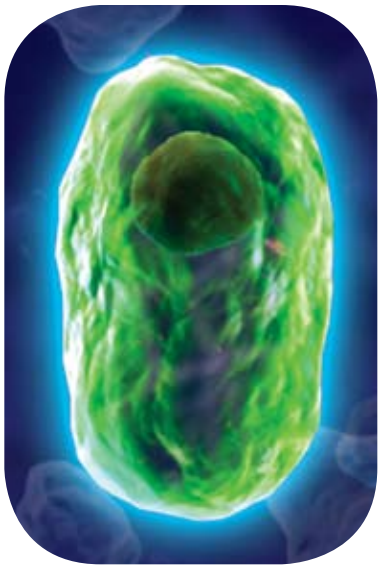


Normal Bone Formation

Normal bone formation and healing involves the coordinated interaction between bone-forming cells and biologic signals. The principal workforce in this process is the osteoblasts and their precursors, the Mesenchymal Stem Cells (MSCs). MSCs are undifferentiated, multipotent cells found in bone marrow, the periosteum, and to a lesser extent, muscle tissue. With proper stimuli, MSCs can differentiate into a number of different cell types, including osteoblasts and chondroblasts. The matrix onto which new bone mineral forms is comprised of a collagen framework that makes up about 30% of human bone. Osteoblasts produce new bone on this collagen matrix, and initiate the release of biologic signals that direct the formation and remodeling of bone. These biologic signals attract MSCs and other bone-forming cells to the site of bone formation as well as cause the differentiation of MSCs into osteoblasts. Mechanical loading of the site can be a type of biologic signal, which directs the activity of these bone-forming cells to produce and remodel new bone. Growth factors and other proteins are also biologic signals that can be involved in new bone formation and healing.



Mesenchymal Stem Cell



Osteoblast

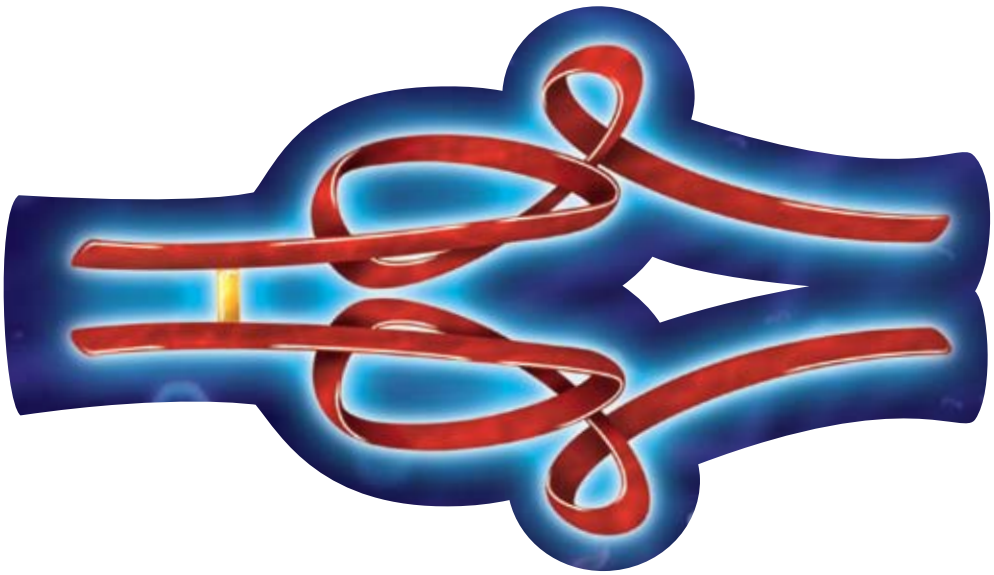
rhBMP-2 Induces New Bone Formation

Bone Morphogenetic Protein

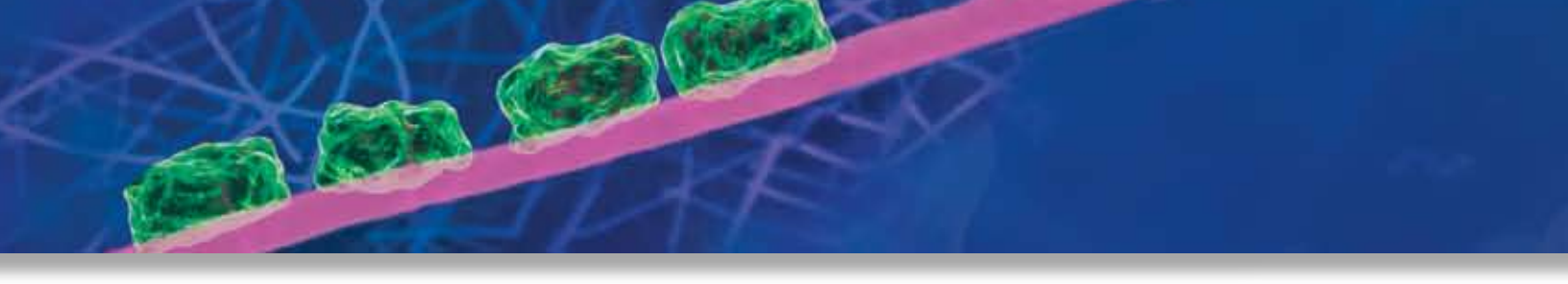
Discovered in 1965 by Dr. Marshall Urist, Bone Morphogenetic Proteins (BMPs) are the only proteins known to induce new bone formation. Dr. Urist used the term Bone Morphogenetic to describe the ability of these bone inductive factors to guide the modulation and differentiation of MSCs into bone and bone-marrow cells.[2] BMPs have been shown to play a role in inducing bone formation during skeletal development[3] and fracture repair.[4] The osteoinductive properties of autologous bone graft and Demineralized Bone Matrix (DBM) are due to the action of BMPs. Pre-clinical experiments from Dr. Urist's lab established that BMPs could be extracted from DBM and still retain the ability to induce new bone formation.[5]

Unique to this family of proteins, BMPs are able to induce MSC differentiation by binding to specific receptors on these cells. More than 20 BMPs have been identified, preclinical animal studies have shown that only certain BMPs are able to induce new bone formation. The ability to stimulate the differentiation of MSCs is the hallmark of the osteoinductive potential of these BMPs.

Through the analysis of protein extracts from bone, BMP-2 has been identified as one of the osteoinductive factors present in bone.[6] BMP-2 has been shown in pre-clinical studies to be capable of inducing MSC migration, proliferation, and differentiation *in-vitro*; and therefore may be involved in every stage of *in-vivo* bone formation. Blum et al, recently measured the amount of BMP-2 from 113 different lots of DBM from various tissue banks and found that the BMP-2 concentration ranged between 200 to 6,744 picograms per gram of DBM.[30] The authors found that the area of new bone formed by these DBM products in a rat ectopic assay was directly proportional to the amount of BMP-2 detected in the DBM.



BMP Molecule



Recombinant Human Bone Morphogenetic Protein

In order to make commercial quantities of BMP, the preferred method is to manufacture a recombinant version of a naturally occurring BMP using well-established molecular biology techniques. This production method results in extremely pure solutions of a single BMP. Recombinant production offers the advantages of tightly controlled manufacturing processes to ensure the consistency, purity, and biologic activity of the final product. Recombinant human Bone Morphogenetic Protein-2 (rhBMP-2, known as dibotermin alfa) is a proven osteoinductive protein and is manufactured using the gene that encodes for human BMP-2.

When an appropriate concentration of rhBMP-2 is placed on an Absorbable Collagen Sponge (ACS) and implanted in the body, it induces new bone tissue at the site of implantation. Histological analyses from several pharmacology studies have characterized the cascade of cellular events involved in the bone induction process initiated by rhBMP-2/ACS (see Table 1). MSCs from the surrounding tissues first infiltrate the rhBMP-2/ACS implant. As the ACS degrades, these MSCs appear to differentiate into bone-forming cells, and begin to form trabecular bone and/or cartilage, with vascular invasion (angiogenesis) evident at the same time (page 20, FDA SS&E, PMA P000058).[1] The bone formation process develops from the outside of the rhBMP-2/ACS implant towards the center until the entire implant is replaced by trabecular bone. Remodeling of the trabecular bone then occurs.

TABLE 1: Mechanism of Action for rhBMP-2/ACS*

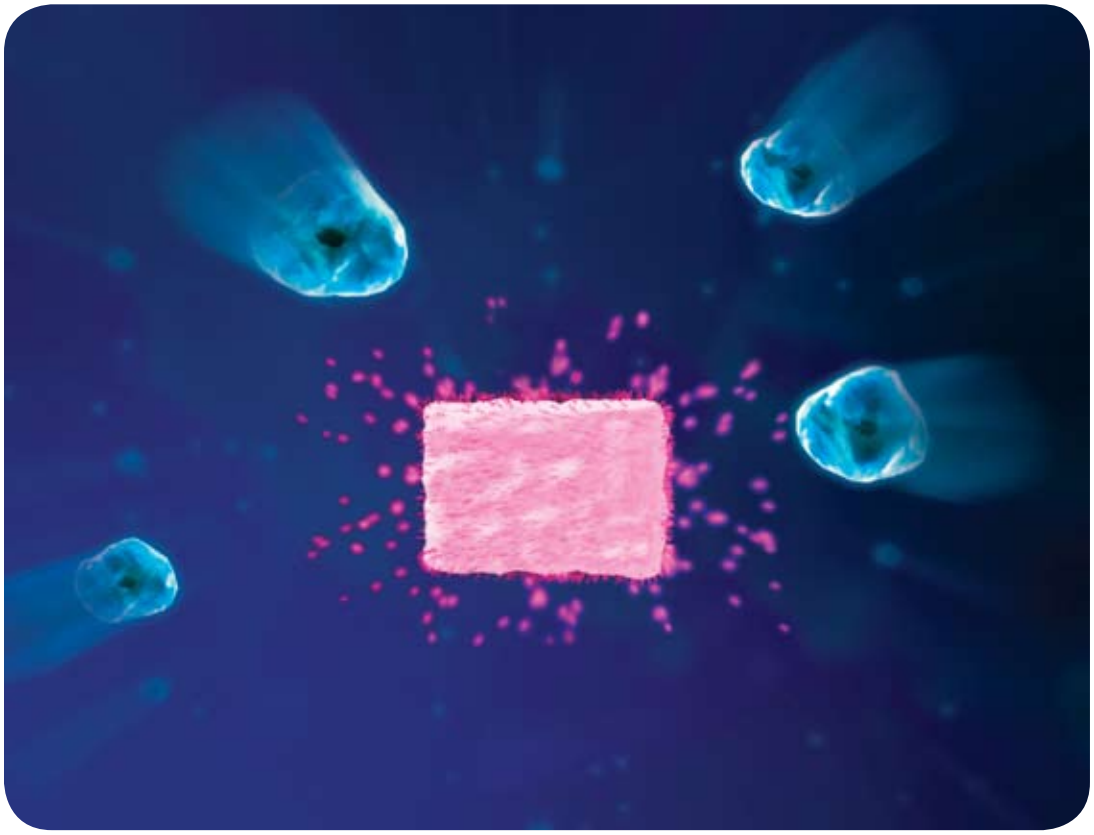
1	Implantation	rhBMP-2/ACS is implanted
2	Chemotaxis	Migration of Mesenchymal Stem Cells and other bone-forming cells to the site of implantation
3	Proliferation	rhBMP-2/ACS provides an environment where stem cells multiply prior to differentiation
4	Differentiation	rhBMP-2 binds to specific receptors on the stem cell surface inducing them to differentiate into osteoblasts
5	Bone Formation and Angiogenesis	Osteoblasts respond to local mechanical forces to produce new mineralized tissue within the ACS. New blood vessel formation is observed at the same time.
6	Remodeling	Body continues to remodel bone in response to the local environmental and mechanical forces, resulting in normal trabecular bone

*** Although the exact mechanism of action in humans is not known, the commonly accepted mechanism of action as determined by *in-vitro* and *in-vivo* animal studies may include the following steps.**

rhBMP-2 Induces New Bone Formation

Chemotaxis

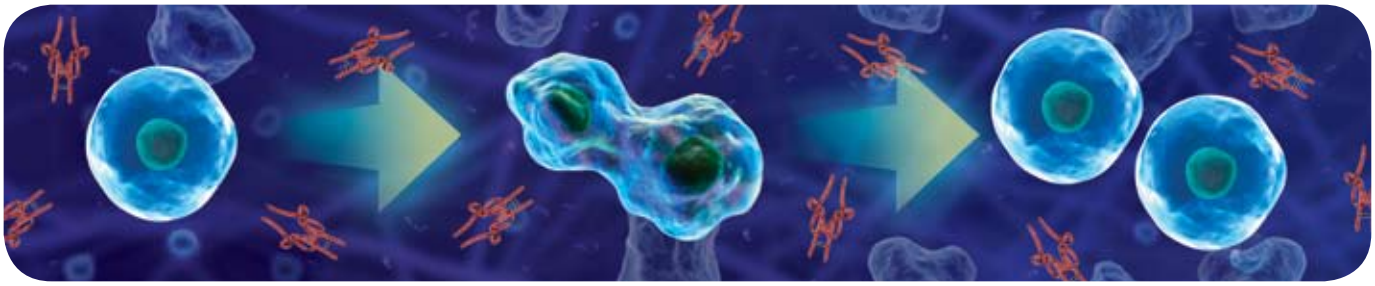
The first step in bone formation process induced by rhBMP-2/ACS, is the migration of bone-forming cells into the area. Chemotaxis is defined as the stimulation of cell migration in response to a chemical signal. MSCs and osteoblasts from bleeding bone, muscle, and the periosteum infiltrate the rhBMP-2/ACS implant. *In-vitro* studies have shown that rhBMP-2 can stimulate the specific chemotactic migration of bone-forming cells.[8-9]



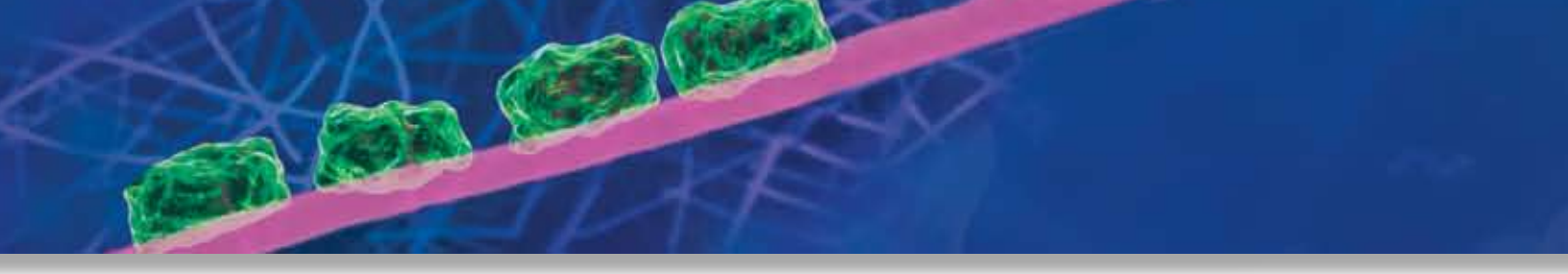
Cells from surrounding tissue infiltrate rhBMP-2/ACS implants.[1]

Proliferation

MSCs proliferate in the vicinity of the rhBMP-2/ACS implantation site. *In-vitro* studies have shown that rhBMP-2 can increase the proliferation of several multipotent cell lines which are capable of differentiating into osteoblasts.[10-14]

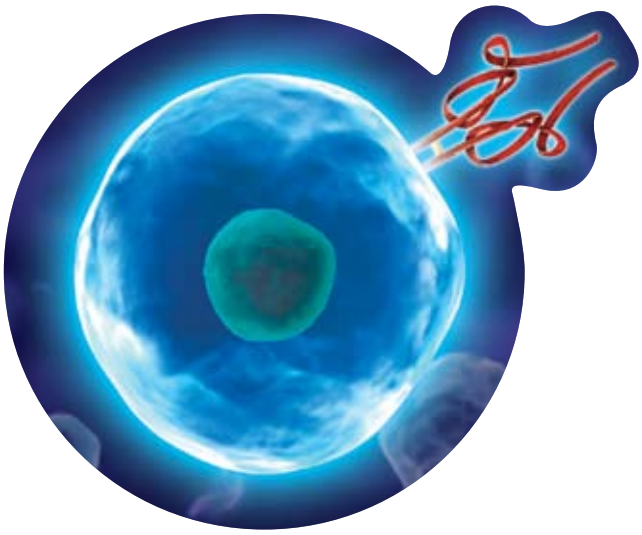


One MSC divides into two MSCs.[1]



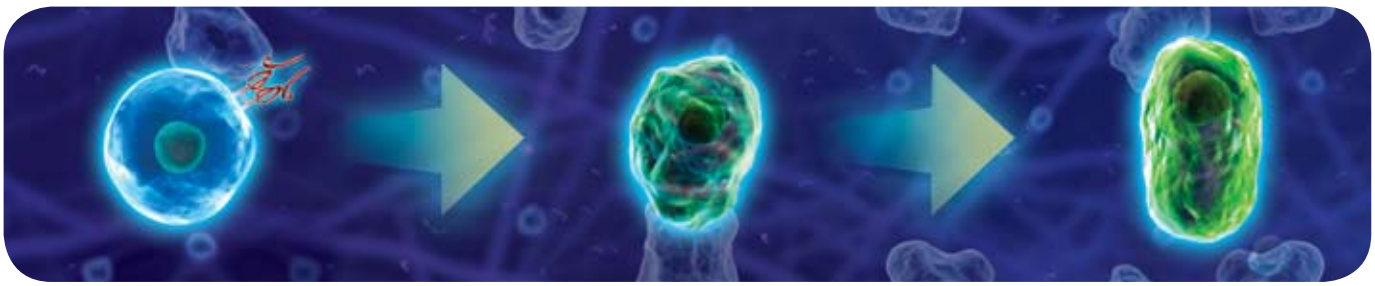
Differentiation

In-vitro studies of rhBMP-2 support the fact that differentiation of MSCs into bone-forming osteoblasts plays an essential role in the induction of new bone.[1] rhBMP-2 binds to specific receptors on the surface of the MSC and causes them to differentiate into bone-forming cells.[4,14] Pre-clinical studies have shown that rhBMP-2 can cause the differentiation of precursor cells into osteoblasts.[10, 12, 14-26, 29, 31]



BMP molecule binding to MSC.[1]

Recently an *in-vitro* study was conducted to compare the osteogenic activity of fourteen recombinant human Bone Morphogenetic Proteins.[27] Three cell lines, representing the different stages of osteoblast differentiation, were each tested. Alkaline phosphatase activity (a measure of the amount of new bone formation) was significantly increased in all three cell lines by BMP-2, 6 and 9. The authors concluded that BMP-2, 6, and 9 may be the most potent agents to induce osteoblast lineage-specific differentiation of MSCs.[27]



MSC + BMP molecule **Pre-osteoblast** **osteoblast**

rhBMP-2 Induces New Bone Formation

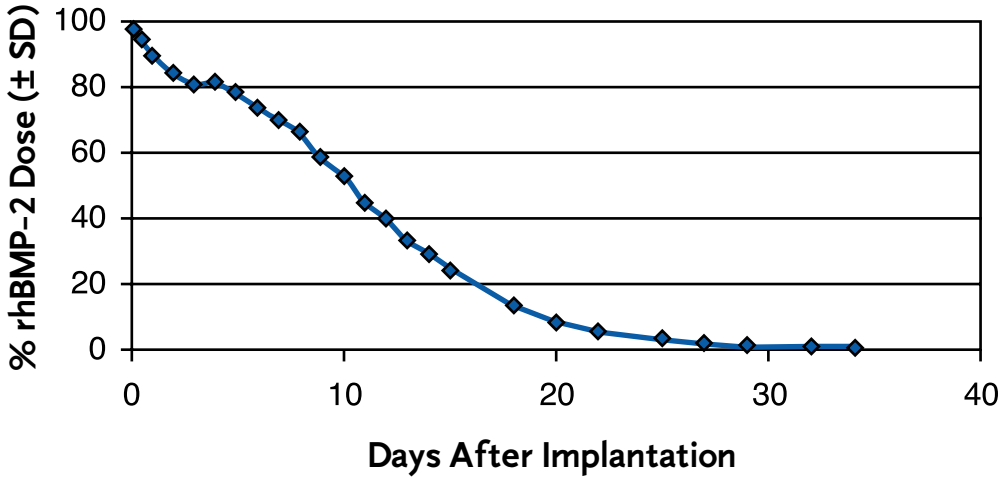
Local, Self-limiting Bone Formation

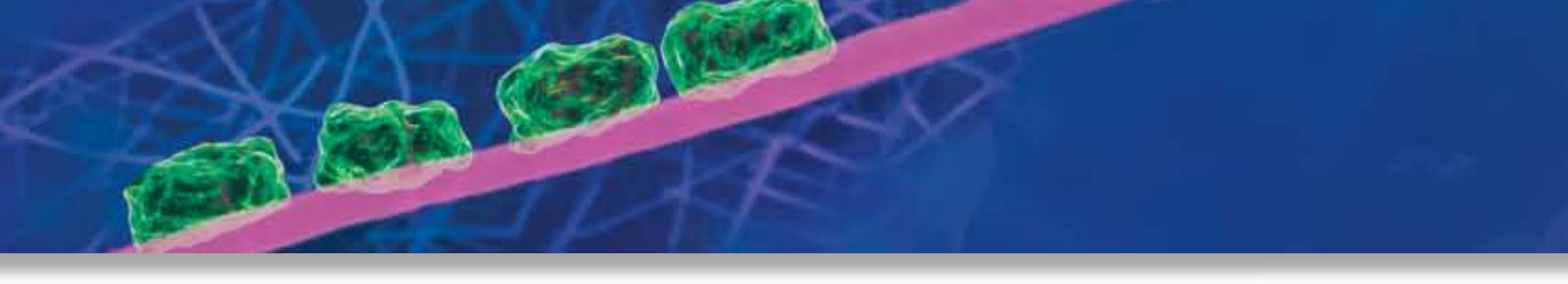
Pre-clinical studies have supported that the bone formation initiated by rhBMP-2/ACS is a self-limiting process, forming a predictable volume of bone. The bone formation process develops from the outside of the rhBMP-2/ACS implant towards the center until the entire implant is replaced by trabecular bone. The ability of rhBMP-2 to induce new bone formation is dependent upon its concentration. The therapeutic rhBMP-2 concentration shifts with the animal species tested in apparent accord with the bone formation rate of that animal. Concentrations below that therapeutic range result in inadequate bone formation. Both the concentration of rhBMP-2 and the length of time that rhBMP-2 is present at the implant site are positively correlated with the rate of bone formation, the amount of bone formed, and the density of the resulting bone (page 20 and 23, FDA SS&E, PMA P000058).[1]

Studies have been conducted to characterize the pharmacokinetics of rhBMP-2 in the blood of rats and monkeys (page 17, 18, and 19, FDA SS&E, PMA P000058).[1] Uptake of rhBMP-2 by highly perfuse tissues and organs is rapid, but the residence time of the protein is short. rhBMP-2 is rapidly eliminated in rat and non-human primates ($t_{1/2}$ = 16 minutes in the rat and $t_{1/2}$ = 6.7 minutes in non-human primates) from systemic circulation following intravenous administration.

Local retention of rhBMP-2 is important to guide localized bone formation. The local residence time of rhBMP-2 when applied to the ACS was assessed following subcutaneous (SC) implantation in rats and implantation at orthotopic sites in rats and rabbits. The results of the three models were similar. In the rat onlay model, ^{125}I-rhBMP-2 was slowly released from the implant site with a mean residence time of approximately eight days (refer to Figure below, page 19, FDA SS&E, PMA P000058).[1]

rhBMP-2 at Implantation Site Following Administration of rhBMP-2/ACS in Rats



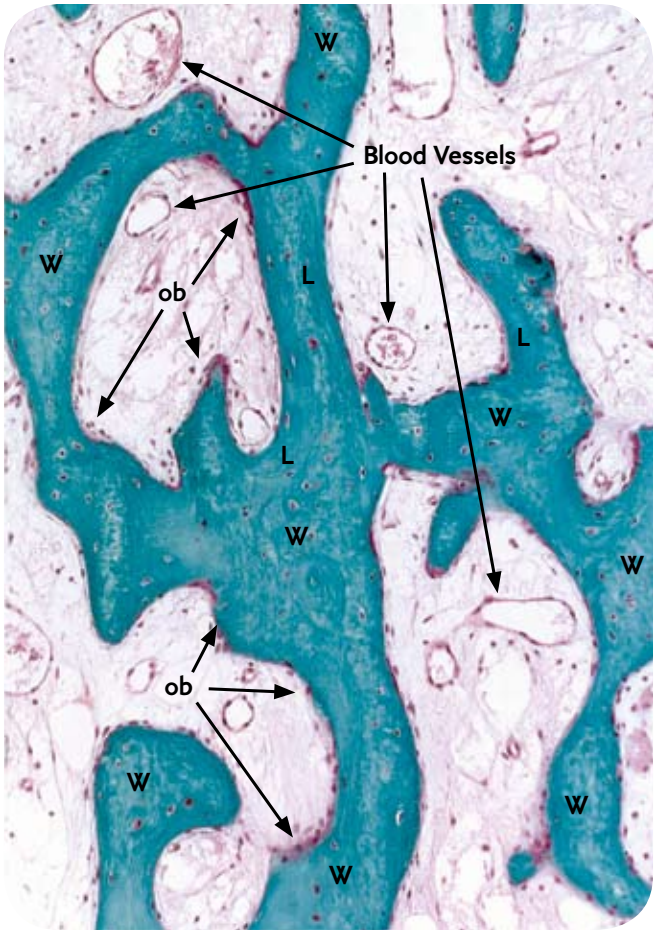


Bone Remodeling & Healing

Remodeling of the trabecular bone induced by rhBMP-2/ACS occurs in a manner that is consistent with the biomechanical forces placed on it. Radiographic, biomechanical, and histologic evaluation of the induced bone indicates that it functions biologically and biomechanically as native bone. Furthermore, pre-clinical studies have indicated that the bone induced by rhBMP-2/ACS can repair itself, if fractured, in a manner indistinguishable from native bone healing (page 20, FDA SS&E, PMA P000058).[1]

Certain clinical studies have allowed for the routine collection of human histology of the new bone induced by rhBMP-2/ACS.[28] Full-width core biopsy specimens obtained 19 to 27 weeks after rhBMP-2/ACS implantation showed actively induced woven and lamellar bone with a moderate to large number of osteoblasts (ob) and capillaries. Qualitative evaluation of these histological samples supports the mechanism of action of rhBMP-2/ACS that results in the induction of new bone formation.

ob = osteoblasts
W = woven bone
L = lamellar bone



Human histology showing the normal bone formed by rhBMP-2/ACS

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BRIEF SUMMARY OF INDICATIONS, CONTRAINDICATIONS, AND WARNINGS FOR:
INFUSE® BONE GRAFT/LT-CAGE® LUMBAR TAPERED FUSION DEVICE
INFUSE® BONE GRAFT/INTER FIX™ THREADED FUSION DEVICE
INFUSE® BONE GRAFT/INTER FIX™ RP THREADED FUSION DEVICE

The INFUSE® Bone Graft/Interbody Fusion Device is indicated for spinal fusion procedures in skeletally mature patients with degenerative disc disease (DDD) at one level from L2-S1, who may also have up to Grade I spondylolisthesis or Grade 1 retrolisthesis at the involved level. The INFUSE® Bone Graft/LT-CAGE® Lumbar Tapered Fusion Device is to be implanted via an anterior open or an anterior laparoscopic approach. INFUSE® Bone Graft with either the INTER FIX™ or INTER FIX™ RP Threaded Fusion Device is to be implanted via an anterior open approach.

The INFUSE® Bone Graft component must not be used without the Interbody Fusion Device component. These components must be used as a system.

NOTE: The INTER FIX™ Threaded Fusion Device and the INTER FIX™ RP Threaded Fusion Device may be used together to treat a spinal level. LT-CAGE® Lumbar Tapered Fusion Device implants are not to be used in conjunction with either the INTER FIX™ OR INTER FIX™ RP implants to treat a spinal level.

The INFUSE® Bone Graft/Interbody Fusion Device is contraindicated for patients with a known hypersensitivity to recombinant human Bone Morphogenetic Protein-2, bovine Type I collagen or to other components of the formulation and should not be used in the vicinity of a resected or extant tumor, in patients with any active malignancy or patients undergoing treatment for a malignancy, in patients who are skeletally immature, in pregnant women, or in patients with an active infection at the operative site or with an allergy to titanium or titanium alloy.

Antibody formation to rhBMP-2 or its influence on fetal development has not been assessed. The safety and effectiveness of this device has not been established in nursing mothers. Women of child-bearing potential should be advised to not become pregnant for one year following treatment with this device.

Please see the package insert for the complete list of indications, warnings, precautions, adverse events, clinical results, definition of DDD, and other important medical information. The package insert also matches the sizes of those sized devices that are indicated for use with the appropriate INFUSE® Bone Graft kit.

CAUTION: Federal (USA) law restricts this device to sale by or on the order of a physician with appropriate training or experience.

BRIEF SUMMARY OF INDICATIONS, CONTRAINDICATIONS, AND WARNINGS FOR: INFUSE® BONE GRAFT

The INFUSE® Bone Graft is indicated for treating acute, open tibial shaft fractures that have been stabilized with IM nail fixation after appropriate wound management. INFUSE® Bone Graft must be applied within 14 days after the initial fracture. Prospective patients should be skeletally mature.

The INFUSE® Bone Graft is contraindicated for patients with a known hypersensitivity to recombinant human Bone Morphogenetic Protein-2, bovine Type I collagen or to other components of the formulation and should not be used in the vicinity of a resected or extant tumor, in patients with an active malignancy or patients undergoing treatment for a malignancy. The INFUSE® Bone Graft should also not be used in patients who are skeletally immature, in patients with an inadequate neurovascular status, in patients with compartment syndrome of the affected limb, in pregnant women, or in patients with an active infection at the operative site.

Antibody formation to rhBMP-2 or its influence on fetal development has not been assessed. The safety and effectiveness of this device has not been established in nursing mothers. Women of child-bearing potential should be advised to not become pregnant for one year following treatment with this device.

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CAUTION: Federal (USA) law restricts this device to sale by or on the order of a physician with appropriate training or experience.

*Commonly accepted mechanisms of action as determined by *in-vitro* and *in-vivo* animal studies. "Application of rhBMP-2/ACS results in the induction of normal bone locally at the site of implantation. This process includes the migration of mesenchymal cells into the site, their proliferation and apparent differentiation into bone-forming cells. The bone induced by rhBMP-2/ACS remodels and assumes the structure appropriate to its location and function, as would be expected from host bone." Page 23, PMA Summary of Safety and Effectiveness Data, INFUSE® Bone Graft/LT-Cage® Lumbar Tapered Fusion Device, PMA Number P000058, July 2, 2002 and Page 20, PMA Summary of Safety and Effectiveness Data, INFUSE® Bone Graft, PMA Number P000054, April 30, 2004. http://www.fda.gov/cdrh/pdf/P000058.html AND http://www.fda.gov/cdrh/pdf4/p000054.html

INFUSE® Bone Graft used in conjunction with LT-CAGE®, INTER FIX™ or INTER FIX™ RP IMPLANTS incorporates technology developed by Gary K. Michelson, M.D.

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